THE ACCESSORY CHROMOSOME IN A FROG POSSESSING MARKED HERMAPHRODITIC TENDENCIES.

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INTRODUCTION.

In the spring of 1916, the writer, while engaged in determining the effects of starvation upon the development of the germ cells and germ glands of larval frogs, noticed an odd chromosomal body in the germ cells of *Rana pipiens*, which simulated the behavior of an accessory chromosome. Owing to the pressure of other work at that time, the matter was neglected until early in the fall of 1916, when a reëxamination of my material led me to believe that the body I had observed the previous spring was probably an accessory or so-called "sex chromosome."

The present paper is a brief preliminary statement of my observations on the peculiar behavior of this body in the germ cells of *Rana pipiens*.

LITERATURE.

So far as the writer is aware, there is little cytological evidence of the presence of an accessory, or sex determining factor in the germ cells of amphibia. Miss King in 1912 described dimorphism in the spermatozoa of *Necturus maculosus* resulting from unequal chromosomal division. This brief paper seems to exhaust the findings upon this particular subject.

There is a great mass of literature dealing with cytological studies of the germ cells of Urodeles, but with the exception of Miss King's paper, inequalities in the distribution of chromatin have never been reported.

King, '07, failed to find any indications of accessory chromosomes in the germ cells of *Bufo*. So far as I am aware, no one seems to have worked with *Rana pipiens*, though the material is excellent in every respect.

Material and Observation.

In the spring of 1916, April 7, to be exact, the writer collected a large number of *Rana pipiens'* eggs from a string of shallow pools near the University campus. The eggs were allowed to develop in the laboratory. The larvæ resulting were used for various experimental purposes, but many were kept until after metamorphosis. Throughout the spring, numbers of the larvæ were killed at odd times up to the period of metamorphosis, in order to obtain a complete series of stages illustrating the normal course of development of the germ glands. It was in the early maturation stages of the germ cells of the older larvæ, that the body simulating the behavior of an accessory chromosome was first observed.

The germ glands of the larvæ were fixed in Flemming's fluid and also potassium-bichromate-acetic; both fixatives gave excellent results. The sections were cut a thickness of 7.5 μ and stained with iron-alum hæmatoxylin. A counter stain of congo red and orange G was used, but equally good results are obtainable without employing counter stains.

Microscopic examination of the preserved germ glands of the older larvæ revealed the odd fact that the animals were of three kinds: males, females, and larvæ indifferent as regards sex; *i. e.*, hermaphrodites, the germ glands of which contained both male and female cells. Richard Hertwig has fully described sexually indifferent frog larvæ, animals potentially capable of developing into either sex; since his paper first appeared, several other investigators have confirmed Hertwig's findings. In *Rana pipiens* this indifferent condition appears to be fairly common, and is especially marked in some larvæ; the animals retaining their bisexual character, even after metamorphosis, when one or other of the two sexes results.

Further examination of my material showed that in the case of the female larvæ, the maturation processes begin early and usually several weeks before metamorphosis; conversely, in the male, the first maturation changes seldom if ever occur until several weeks after metamorphosis. All stages of the early maturation processes of the germ cells are visible in the glands of female larvæ seven to eight weeks of age.

The oögonial number of chromosomes in the female larvæ appears to be twenty-six. It was possible to make, however, only a few counts, and this number stands, therefore, subject to revision. The male spermatogonial counts will be discussed later. Two oögonial counts were made from a specimen of *Rana catesbiana*, which also gave twenty-six chromosomes as the diploid number. (See Fig. 1.) The size and shape of the chromosomes in the two species of frog appear very similar.

In the post-synizesis stages of the germ cells of the female larvæ of *Rana pipiens*, the chromatin threads which appear from the dense, tangled, heavily staining contraction figure (Figs. 2 and 3), are thick, beaded structures, which have a marked tendency to arrange themselves into the well-known bouquet figures. At this stage all of the chromatin has become arranged in the form of pachytene threads, except a rather large, heavily staining body usually found adhering closely to the nuclear wall (Figs. 4–6). This body is somewhat irregular in shape, though constant in size; the shape usually assumed is that of a triangle, with a minute, thread-like structure at the apex. In the bouquet figures, this chromatin mass orients itself toward one pole of the cell along with the free ends of the pachytene threads (Fig. 6).

Study of a somewhat similar body in the adult male indicates that the body observed in the germ cells of the female larvæ represents probably the accessory chromosomes. At the stage shown in Figs. 4 and 6, the body appears double in most cells. In other cells, for instance Fig. 5, the body is shown as single. Attempts to trace this structure back through the synizesis stage proved futile in my *Rana pipiens* material, owing to certain stains employed by me in connection with a different problem, but in specimens of *Rana catesbiana* larvæ 70 mm. in length, pre-synizesis stages reveal a structure apparently identical with the post-synizesis X-body of *Rana pipiens*.

In female *Rana pipiens*, soon after the synaptic stages of the maturation process occur, the young oöcytes are formed and further study of the *X*-body is impossible in the larval form. Attention was then turned to the germ glands of young male frogs.

Through the kindness of Dr. B. M. Allen, I obtained a number

of sections of the testes of young frogs killed several weeks after metamorphosis had occurred. One of these young frogs was a pseudo-hermaphrodite, over a year old. The testes of this animal contained both ripe spermatozoa and large oöcytes. The germ glands, though containing many oöcytes, were true testes; *i. e.*, the animal was a modified male. All stages of the maturation process were visible; in many follicles the oöcytes and ripe spermatozoa occurred side by side.

The sections had been stained with hæm-alum, counter stained with eosin when received by me. This stain serves very well for the maturation divisions, but is not to be recommended for spermatogonial counts, as it renders the outlines of the chromosomes hazy. As a consequence of this, all of my spermatogonial counts were obtained from sections of testes from other animals, stained with iron-alum hæmatoxylin.

Ten or twelve spermatogonial counts gave twenty-five chromosomes as the diploid number. (See Figs. 7–9.) The chromosomes vary greatly in size and shape, from large V-shaped bodies to small straight rods.

In the hermaphrodite specimen a great many prophases of the first spermatocyte division were found. The chromosomes at this stage were undergoing reduction in number, and appeared as thirteen ring and dumb-bell-shaped bodies of varying size. (See Figs. 10–12.) At slightly later stages, the rings assume the dumb-bell shape also, and are connected end to end by fine linin threads. Still later stages show the linin connectives breaking and the chromosomes becoming scattered through the nucleus. There are thirteen chromosomes visible in the cells. Near the end of the early prophase period, twelve of the typical dumb-bell-shaped prophase chromosomes round off somewhat, becoming oval-shaped; one of the dumb-bell bodies, however, retains its shape; this body, because of its peculiar behavior, I have termed the *X*-body. It is not difficult to identify at this stage because of its large size and dumb-bell shape (Fig. 13).

During the early metaphase stages of the first spermatocyte division, the prophase chromosomes, now oval-shaped, except the *X*-body, line up in the equatorial region of the cell to form the metaphase plate. The *X* chromosome takes its position on

the metaphase plate along with the other chromosomes. The X-body is readily detected at this stage by reason of its marked dumb-bell appearance.

Shortly before the chromosomes split, the dumb-bell-shaped X-body migrates to one or other pole of the cell, far in advance of the other chromosomes, and comes to lie at the apex of the spindle close to the centrosome (Figs. 14–19). Lateral views of such spindles, with the chromosomes at the metaphase, are difficult to count; the larger chromosomes obscure the smaller in many instances. Usually, however, twelve obviously bivalent chromosomes can be counted at this stage, in the equatorial region of the cell, just previous to splitting of the chromosomes, and one large, dumb-bell-shaped X-body, the accessory, at one or the other pole.

The resulting division separates the twelve bivalents into halves, each half migrating toward its respective pole. As a result of this division, an unequal distribution of chromatin to the secondary spermatocytes occurs; one cell receiving twelve chromosomes plus the dumb-bell X, the other cell receiving twelve ordinary chromosomes.

A late anaphase of the first spermatocyte division, showing the X-body at one pole of the anaphase, the other pole without the accessory, is shown in Fig. 20.

The dumb-bell X-body is very conspicuous in many cells in late anaphase stages. It retains the dumb-bell shape, whereas the remainder of the chromosomes are comparatively small, single bodies. In late telophase stages of the first maturation division, all individuality of the chromosomes is lost; the chromosomes clumping together to form a somewhat irregular crescent. In many such stages the accessory chromosome is easily located by the fact that it is so large that half of it projects from the chromatin mass. It may lie horizontal to the long axis of the cell, resting on the other chromosomes, or at times partly imbedded within their mass.

Three first spermatocyte divisions were observed in which the X-body appeared as a single and not a dumb-bell-shaped chromosome. Figs. 21–23 show the single nature of the X chromosome. In these cells, the X is just half the size it appears in other cells.

At the side of the metaphase plate opposite from the X-body in one of the three cells, another single body, evidently the product of a precocious division, was observed. This is shown in Fig. 23. In this cell, the dumb-bell accessory seems to have divided, the halves passing to opposite poles. This explanation may also hold for the other two cells in which the X-body appears single, though the other half was not observed. There is a possibility that the single appearance of the X may be due to end views of the body, though such a possibility seems slight. The very large size of the X would, in my opinion, preclude any such possibility.

The secondary spermatocytes, resulting from the division of one of these three cells, the cell in which the X-body divided (Fig. 23), would each receive thirteen chromosomes, each cell receiving half of the accessory; whereas, in the case of the remaining two cells, one half the second spermatocytes would receive thirteen chromosomes, the other half, twelve.

Three other cases of dividing first spermatocytes were observed, in which the typical dumb-bell-shaped X-body had migrated very early to one pole of the cell, and at the other pole a large, round chromosome appeared.

The two parts of the X-body in one cell, were unequal in size, one being considerably smaller than the other. (See Figs. 24–26.) The single, round chromosomes at the opposite pole from the X-body in Fig. 24, judging from its size and shape, appears to be the true half of the accessory or X.

The behavior of the chromosomes in these three cells is difficult to explain satisfactorily. It is obvious, however, that the secondary spermatocytes resulting from such divisions would receive unequal amounts of chromatin.

Repeated examination of my material convinces me that the normal distribution of chromatin to the secondary spermatocytes, at the first maturation division, is twelve chromosomes, plus the dumb-bell-shaped X to one cell, and twelve ordinary chromosomes to the other.

In regard to the case of those cells just described in which the X-body appears to have divided precociously, half passing to each pole in advance of the other chromosomes, it is probable

that we are dealing with abnormal divisions. The same is true, of course, of those cells in which the accessory either divided precociously, half passing to each pole, but one half becoming linked with a smaller chromosome or perhaps a chromatoid body (see Fig. 24), or else the halves of the X are unequal.

However this may be, it should not be forgotten that the animal in whose germ cells these anomalies of chromosomal distribution occur, is bisexual; *i. e.*, with the germinal products of both sexes in its glands. There may, or may not be, a connection between such abnormalities of chromatin distribution at the maturation divisions, resulting, presumably, in the production of three kinds of spermatozoa, and the fact that in certain strains of this species of frog, males, females and animals possessing marked hermaphroditic tendencies occur.

It is obvious, however, that the presence of oöcytes in my specimen is not due to any inequality in chromatin sharing of the daughter cells at the maturation divisions, because the oöcytes are formed long before the male maturation period occurs. Such oöcytes may be due to unequal chromosomal division at an earlier period; perhaps in the spermatogonia or even primordial germ cells. In this connection it may be mentioned that in those follicles containing oöcytes, and they are far from uncommon in my specimen, spermatids and mature spermatozoa are also usually found. The two kinds of cells may be found side by side. The fact that the germinal products of both sexes are usually found together in the same follicle suggests that perhaps both oöcytes and spermatozoa may be products of the same cell originally. It might not be amiss to point out here that the oöcytes and spermatozoa found in the same follicles of this animal represent advanced stages in the germ cell cycle, not found in the surrounding follicles. The surrounding follicles contain spermatogonia and prophases of first maturation divisions, stages much younger than spermatozoa and egg formation.

The Second Spermatocytes.

The second spermatocytes resulting from the first maturation division divide at once without an observable period of rest. The telophase of the first spermatocyte division leaves the chro-

matin matter a solid, somewhat irregular crescentic mass. This mass soon rounds off its angularities and arranges itself along the equatorial region of the cell. The outlines of the individual chromosomes are, for the most part, obliterated at this stage. No further reduction of chromosomes occurs at this division. The dumb-bell-shaped *X*-body is not difficult to identify in the second spermatocyte at the metaphase. Its identification is rendered easy by its large size and peculiar dumb-bell shape. (See Figs. 27–28.) Part of the dumb-bell usually is seen projecting above the metaphase plate. This is well shown in Figs. 27–28.

All attempts to determine the chromosomal number in secondary spermatocytes in my bisexual specimen proved futile, owing to the hæm-alum stain. The dumb-bell *X*-body divides along with the rest of the second spermatocyte chromosomes. No attempt was made to trace the further history of the accessory body in the spermatids and spermatozoa. I hope to take up this matter in another paper.

The Chromatoid Body.

A number of investigators of amphibian spermatogenesis have observed and reported the presence of an oval, homogeneous chromatoid body in the cytoplasm of the germ cells.

King, '07, reported it in *Bufo*, and claims it forms the acrosome of the spermatozoon. Herman has found a similar body in the spermatids of *Salamandra*. Recently Bachhuber reported the presence of such a body in the spermatocytes of the rabbit. It has also been reported for other animals.

In *Rana pipiens* the chromatoid body is readily found in the cytoplasm of the secondary spermatogonia. I have never observed it in the primordial germ cells of very young larvæ. This structure is of fairly large size, oval in shape and stains readily. It bears considerable resemblance to an extruded nucleolus, though probably has an extra-nuclear origin. The position of the body in the cytoplasm varies considerably. In some cells it adheres closely to the nuclear wall; in other cells it is found near the periphery of the cell. During spermatogonial division it does not appear to divide, though it occurs among the

spindle fibres; actual division of the body was observed but once. (See Fig. 29.) The body is readily distinguished from the chromosomes by its peculiar character, and the position it takes during cell division.

In the primary spermatocytes the chromatoid body is conspicuous in the cytoplasm. Whether it does or does not divide at the first maturation division was not ascertained. In Fig. 24 it is shown passing to one pole, apparently undivided.

I make mention of the behavior of this structure here, in order to disarm, beforehand, the criticism which perchance might arise in connection with this paper, that I may have mistaken the chromatoid body for an accessory chromosome. The origin and fate of this peculiar body in *Rana pipiens* seem worthy of further investigation.

Summary and Conclusion.

1. The oögonial number of chromosomes in *Rana pipiens* and *catesbiana* appears to be twenty-six.

2. The spermatogonial number is twenty-five in *Rana pipiens*.

3. In the synaptene stage of the germ cells of the female *Rana pipiens* larvæ, a chromatin body is found which simulates the behavior of an accessory chromosome.

4. The reduced number of chromosomes in the male of *Rana pipiens* is thirteen.

5. At the first maturation division of the germ cells of a frog possessing marked hermaphroditic tendencies, there is an unequal distribution of chromatin to the daughter cells, the inequality varying in different cells.

6. Perhaps this unequal distribution of chromosomes at the spermatocyte division and the resulting inequality of chromosomal distribution to the spermatozoa may account for the fact that males, females, and animals possessing marked hermaphroditic tendencies are found in this species of frog.

In conclusion, I wish to acknowledge my indebtedness to Dr. B. M. Allen for the loan of several valuable slides and to Dr. W. R. B. Robertson for several excellent suggestions.

April 11, 1917.

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EXPLANATION OF FIGURES ON PLATES I–VI.

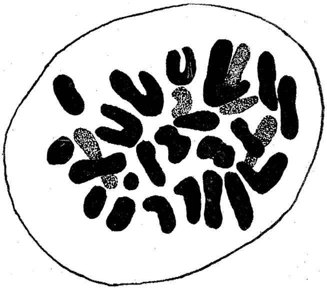
All figures were drawn with the aid of a camera lucida. Spencer 2 mm. apochromat oil immersion objective and oc. 18 used. Drawings are accurate with reference to the nuclear material and chromatoid body. The cytoplasm, however, is represented in the conventional way. *ch*, chromatoid body; *C*, centrosome.

PLATE I.

FIG. 1. Polar view of metaphase plate in dividing oogonia from ovary of *Rana catesbiana*, twenty-six chromosomes are present.

FIGS. 2 and 3. Synizesis stages in young oöcytes of *Rana pipiens* larvæ.

FIGS. 4, 5 and 6. Young oöcytes of *Rana pipiens*, showing the pachytene threads and the X-chromosome adhering to the nuclear wall.



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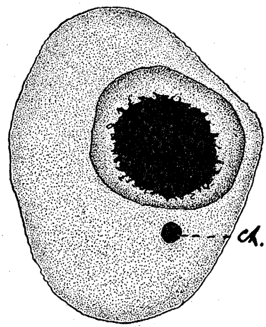

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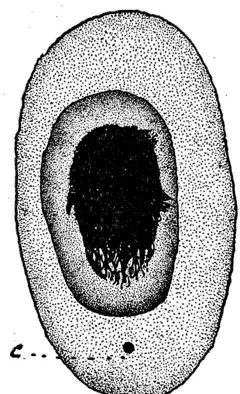

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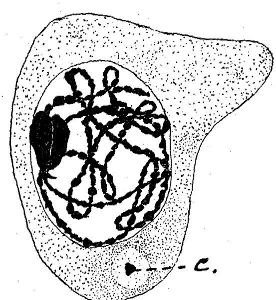

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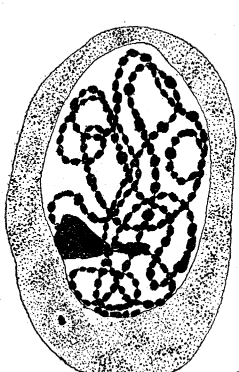

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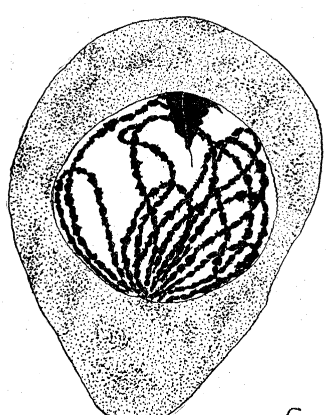

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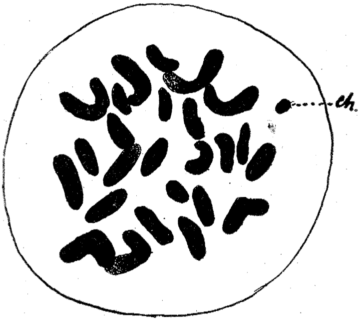
PLATE II.

FIGS. 7, 8 and 9. Spermatogonia of *Rana pipiens*, showing twenty-five chromosomes.

FIGS. 10, 11, 12 and 13. Spermatocytes, showing prophases of the first maturation division.



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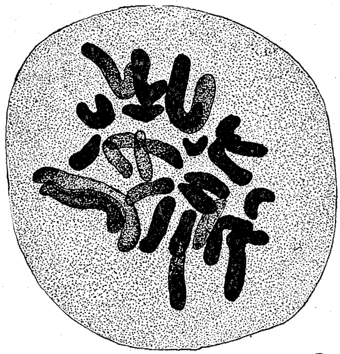

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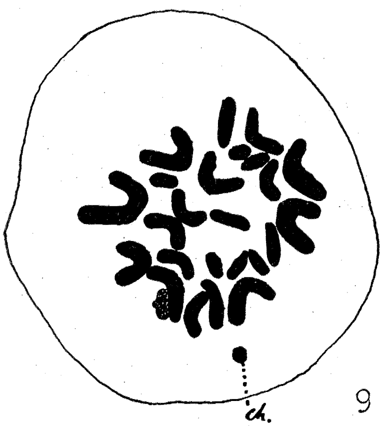

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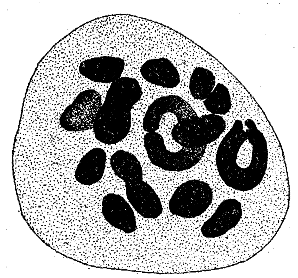

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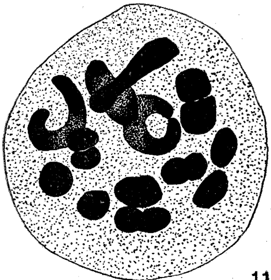

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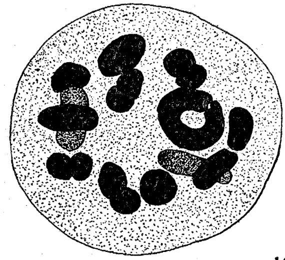

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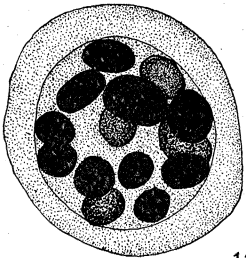
PLATE III.

FIGS. 14, 15, 16, 17, 18 and 19. Spermatocytes, showing first maturation division and the migration of the accessory chromosomes to one pole.



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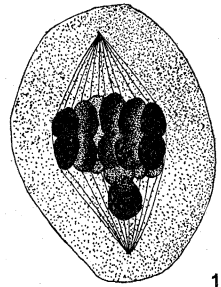

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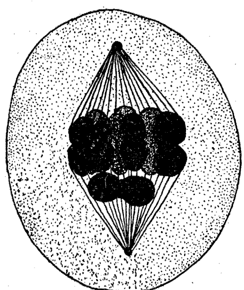

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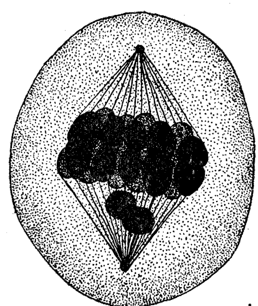

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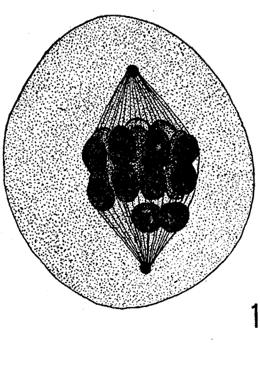

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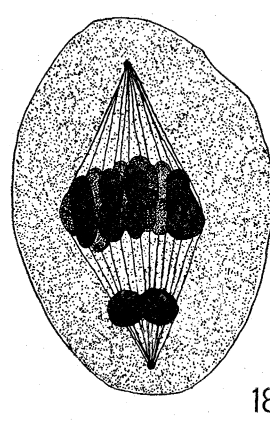

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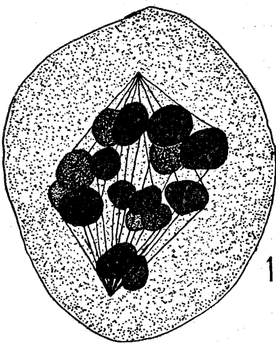
PLATE IV.

FIG. 20. Late anaphase of a dividing first spermatocyte showing the accessory chromosome at one pole.

FIGS. 21, 22 and 23. First maturation divisions showing single nature of accessory chromosome.

FIG. 24. First maturation division showing accessory chromosome at one pole and single round chromosome at opposite pole.



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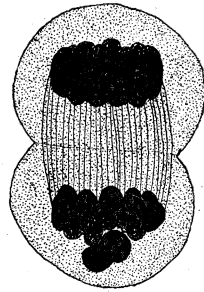

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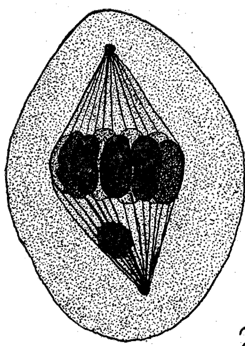

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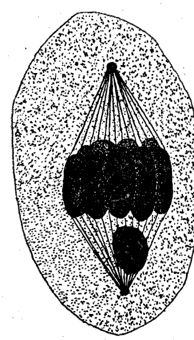

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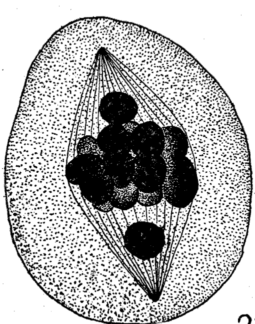

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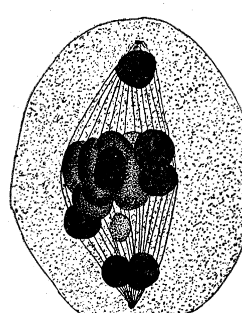

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PLATE V.

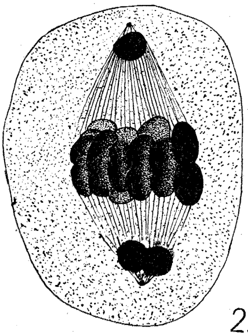
FIGS. 25 and 26. First maturation divisions showing accessory chromosome at one pole of the cell; single chromosome at opposite pole.

FIGS. 27 and 28. Second spermatocytes showing the large accessory chromosome.

FIG. 29. Anaphase of dividing spermatogonium showing division of the chromatoid body.

FIG. 30. Spermatocyte of *Rana catesbiana* with two chromatoid bodies in the cytoplasm.

FIG. 31. Spermatogonium of *Rana pipiens* showing large chromatoid body.



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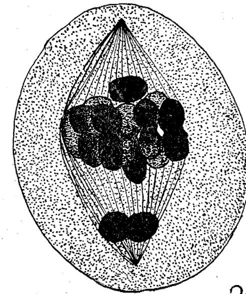

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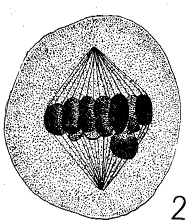

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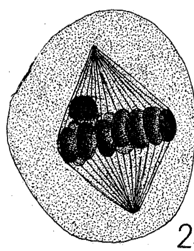
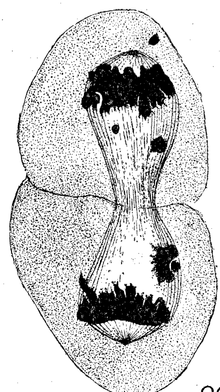


28



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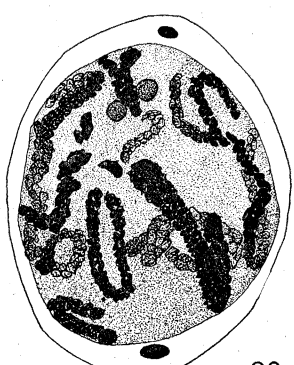
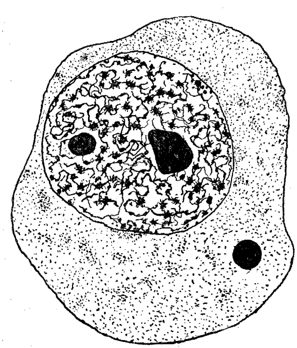


30

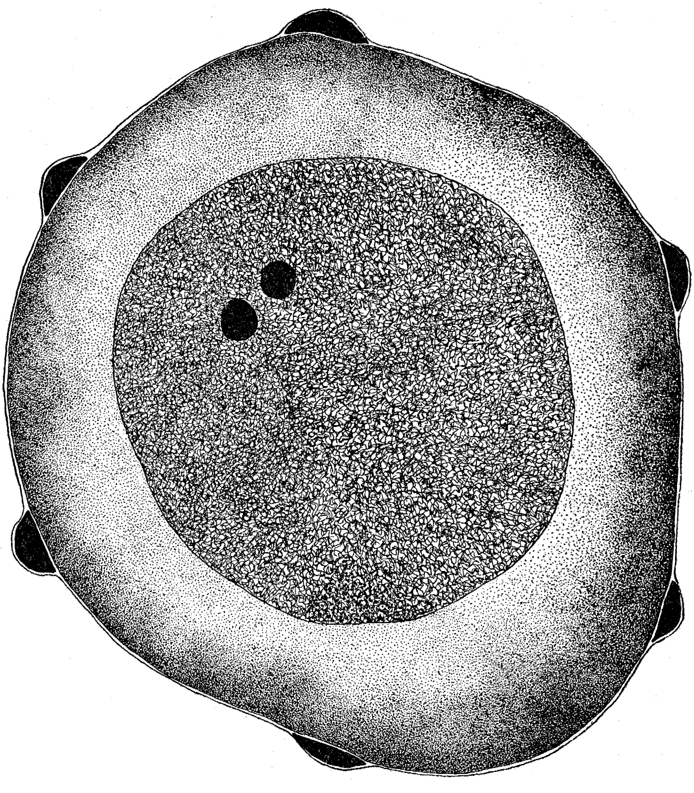


31

W. W. SWINGLE.

PLATE VI.

FIG. 32. Oöcyte from the testis of young frog



32

W. W. SWINGLE.